Attorney Docket No: 9435-2

What is claimed is:

1. An isolated nucleic acid comprising a single retroviral long terminal repeat (LTR), a polypurine tract, a packaging signal, a primer binding site and a rev responsive element.

- 2. The nucleic acid of claim 1, further comprising a central polypurine tract.
- 3. The nucleic acid of claim 1, further comprising a post-transcriptional regulatory element.
- 4. A vector comprising the nucleic acid of claim 1.
- 5. An isolated nucleic acid comprising a heterologous nucleotide sequence, a single retroviral long terminal repeat (LTR), a packaging signal, a rev responsive element, a polypurine tract, a eukaryotic promoter, a primer binding site, a bacterial origin of replication and a bacterial selection marker.
- 6. The nucleic acid of claim 5, further comprising a central polypurine tract.
- 7. The nucleic acid of claim 5, further comprising a post-transcriptional regulatory element.
- 8. A vector comprising the nucleic acid of claim 5.
- 9. The nucleic acid of claim 5, wherein a major portion of the U3 region of the LTR is deleted.
- 10. The nucleic acid of claim 9, wherein the portion of the U3 region that has been deleted is replaced with an inducible promoter.
- 11. The nucleic acid of claim 5, wherein the U3 region of the LTR comprises a loxP site.

- 12. The nucleic acid of claim 5, wherein the U3 region of the LTR comprises a restriction site.
- 13. An isolated nucleic acid comprising a 5' retroviral LTR and a 3' retroviral LTR, a heterologous nucleotide sequence, a packaging signal, a rev responsive element, a polypurine tract, a eukaryotic promoter, a primer binding site, a bacterial origin of replication and a bacterial selection marker, wherein the bacterial origin of replication and bacterial selection marker are located between the two LTRs.
- 14. The nucleic acid of claim 13, further comprising a central polypurine tract.
- 15. The nucleic acid of claim 13, further comprising a post-transcriptional regulatory element.
- 16. The nucleic acid of claim 13, wherein a major portion of the U3 region of the LTR is deleted.
- 17. The nucleic acid of claim 16, wherein the portion of the U3 region that has been deleted is replaced with an inducible promoter.
- 18. The nucleic acid of claim 13, wherein the U3 region of the 3' LTR comprises a *loxP* site.
- 19. The nucleic acid of claim 13, wherein the U3 region of the LTR comprises a restriction site.
- 20. A method of producing a single-LTR circular HIV-1 form plasmid, comprising
 - a. introducing the nucleic acid of claim 5 into a eukaryotic cell;
 - b. extracting non-integrated DNA from the eukaryotic cell;
 - c. transforming a bacterial cell with the DNA of step (b);

d. selecting a bacterial cell showing expression of a selection marker; and isolating a single-LTR circular HIV-form plasmid from the bacterial cell.

- 21. A method of making a retroviral vector particle, comprising:
 - a) introducing the vector of claim 8 into a retroviral packaging cell in medium, said packaging cell comprising nucleotide sequences encoding rev, gag/pol and env proteins but lacking packaging sequences; and
 - b) collecting retroviral vector particles from the medium.
- 22. A method of producing a retroviral expression vector, comprising cloning the nucleic acid of claim 1 into a non-retroviral expression vector.
- 23. A retroviral expression vector produced by the method of claim 21.
- 24. A method of isolating a cDNA sequence that encodes a gene product that results in a particular phenotype upon contact with a test substance, comprising:
 - a. producing a cDNA library in a population of nucleic acids of Claim 11;
 - b. introducing the nucleic acids of step (a) into eukaryotic cells;
 - c. contacting the cells of step (b) with the test substance;
 - d. introducing a nucleic acid encoding Cre protein into surviving cells of step (c) under conditions whereby the Cre protein nucleic acid is expressed;
 - e. extracting circular DNA from the cells of step (d);
 - f. transforming a bacterial cell with the circular DNA of step (e); and
 - g. isolating from the bacterial cell the cDNA sequence that encodes a gene product that results in a particular phenotype upon contact with a test substance.
- 25. The method of Claim 24, wherein the cDNA library is produced by cloning an isolated nucleic acid comprising a single retroviral long terminal repeat (LTR),

- a polypurine tract, a packaging signal, a primer binding site and a rev responsive element into a non-retroviral cDNA library.
- 26. The method of Claim 24, wherein the U3 region of the LTR comprises a restriction site.
- 27. A method of isolating a cDNA sequence that encodes a gene product that results in a particular phenotype upon contact with a test substance, comprising:
 - a. producing a cDNA library in a population of nucleic acids of Claim 18;
 - b. introducing the nucleic acids of step (a) into eukaryotic cells;
 - c. contacting the cells of step (b) with the test substance;
 - d. introducing a nucleic acid encoding Cre protein into surviving cells of step (c) under conditions whereby the Cre protein nucleic acid is expressed;
 - e. extracting circular DNA from the cells of step (d);
 - f. transforming a bacterial cell with the circular DNA of step (e); and
 - g. isolating from the bacterial cell the cDNA sequence that encodes a gene product that results in a particular phenotype upon contact with a test substance.
- 28. The method of Claim 27, wherein the cDNA library is produced by cloning an isolated nucleic acid comprising a single retroviral long terminal repeat (LTR), a polypurine tract, a packaging signal, a primer binding site and a rev responsive element into a non-retroviral cDNA library.
- 29. The method of Claim 27, wherein the U3 region of the LTR comprises a restriction site.